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(54) Title: INHIBITORS OF GOB-4 PROTEIN AS ASTHMA THERAPEUTICS

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1 cccgcatccta gccgcccgaact cacacaaggc aggtgggtga ggaaatccag agttgccatg
61 gagaaaaattc cagtgtcagc attcttgctc cttgtggccc tctcctacac tctggccaga
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1501 gaattgtgtg ttaatgtctt cactctatac ttaatctcac attcattaat atattggaatt
1561 cctctactgc ccagcccctc ctgatttctt tggccctctg actatggtgc tgtatataat
1621 gctttgcagt atctgttgct tctcttgatt aacttttttg gataaaacct tttttgaaca
1681 gaaaaaaaaa aaaaaaaaaa a

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(57) Abstract: Methods of screening for agents for treating asthma are provided. The methods involve screening for agents that decrease the production or activity of a Gob-4 protein that has been discovered herein to play a role in producing the symptoms and pathological complications involved in asthma. Methods of treating asthma, as well as screening for and treating with inhibitors of a Gob-4 protein are also provided.



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INHIBITORS OF GOB-4 PROTEIN AS ASTHMA THERAPEUTICS

FIELD OF THE INVENTION

5 It is an object of the invention to provide methods of screening for agents for treating asthma. It is a further object of the invention to provide methods for treating asthma. These and other objects and advantages of the present invention will be apparent from the descriptions herein.

BACKGROUND OF THE INVENTION

10 [0001] The present invention relates generally to asthma therapeutics. Specifically, the invention relates to methods of screening for agents for treating asthma and methods for treating asthma.

[0002] Asthma is a chronic inflammatory disease of the airways characterized by recurrent episodes of reversible airway obstruction and airway
15 hyperresponsiveness (AHR). Typical clinical manifestations include shortness of breath, wheezing, coughing and chest tightness that can become life threatening or fatal. While existing therapies focus on reducing the symptomatic bronchospasm and pulmonary inflammation, there is a growing awareness of the role of long term airway remodeling in accelerated lung deterioration in asthmatics. Airway
20 remodeling refers to a number of pathological features including epithelial smooth muscle and myofibroblast hyperplasia and/or metaplasia, subepithelial fibrosis and matrix deposition. The processes collectively result in up to about 300% thickening of the airway in cases of fatal asthma. Despite the considerable progress that has been made in elucidating the pathophysiology of asthma, the
25 prevalence, morbidity, and mortality of the disease has increased during the past two decades. In 1995, in the United States alone, nearly 1.8 million emergency room visits, 466 thousand hospitalizations and 5,429 deaths were directly attributed to asthma.

[0003] It is generally accepted that allergic asthma is initiated by an inappropriate inflammatory reaction to airborne allergens. The lungs of asthmatics demonstrate an intense infiltration of lymphocytes, mast cells and especially eosinophils. A large body of evidence has demonstrated that this immune response is driven by CD4⁺ T-cells expressing a T_H2 cytokine profile. One murine model of asthma involves sensitization of the animal to ovalbumin (OVA) followed by intratracheal delivery of the OVA challenge. This procedure generates a T_H2 immune reaction in the mouse lung and mimics four major pathophysiological responses seen in human asthma, including upregulated serum IgE (atopy), eosinophilia, excessive mucus secretion, and AHR. The cytokine IL-13, expressed by basophils, mast cells, activated T cells and NK cells, plays a central role in the inflammatory response to OVA in mouse lungs. Direct lung instillation of murine IL-13 elicits all four of the asthma-related pathologies and, conversely, the presence of a soluble IL-13 antagonist (sIL-13R α 2-Fc) completely blocked both the OVA-challenge induced goblet cell mucus synthesis and the AHR to acetylcholine. Wills-Karp, M., *et al.*, "Interleukin-13: central mediator of allergic asthma," *Science* **282**(5397): 2258-2261 (1998); Grunig, G., *et al.*, "Requirement for IL-13 independently of IL-4 in experimental asthma," *Science* **282**(5397): 2261-2263 (1998). Thus, IL-13 mediated signaling is sufficient to elicit all four asthma-related pathophysiological phenotypes and is required for the hypersecretion of mucus and induced AHR in the mouse model.

[0004] Biologically active IL-13 binds specifically to a low-affinity binding chain IL-13R α 1 and to a high-affinity multimeric complex composed of IL-13R α 1 and IL-4R, a shared component of IL-4 signaling complex. Wills-Karp, M., "IL-12/IL-13 axis in allergic asthma," *J Allergy Clin Immunol* **107**(1): 9-18 (2001). Activation of the IL-13 pathway cascade triggers the recruitment, phosphorylation and ultimate nuclear translocation of the transcriptional activator Stat6. A number of physiological studies demonstrate the inability of pulmonary OVA-challenge to elicit major pathology related phenotypes including eosinophil infiltration, mucus hypersecretion and airway hyperreactivity in mice homozygous for the Stat6^{-/-} null allele. Kuperman, D., *et al.*, "Signal transducer and activator of

transcription factor 6 (Stat6)- deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production,” *J Exp Med* **187**(6): 939-48 (1998). Recent genetic studies have demonstrated a linkage between specific human alleles of IL-13 and its signaling components with asthma and atopy, demonstrating the critical role of this pathway in the human disease. Shirakawa *et al.*, “Atopy and asthma: genetic variants of IL-4 and IL-13 signaling,” *Immunol. Today* **21**(2):60-64 (2000).

[0005] IL-13 also binds to an additional receptor chain, IL-13R α 2, expressed in both human and mouse with as yet undefined biological function. The murine IL-13R α 2 binds IL-13 with approximately 100-fold greater affinity (Kd of 0.5 to 1.2nM) relative to IL-13R α 1, allowing the construction of a potent soluble IL-13 antagonist, sIL-13R α 2-Fc. The sIL-13R α 2-Fc has been used as an antagonist in a variety of disease models to demonstrate the role of IL-13 in Schistosomiasis induced liver fibrosis and granuloma formation, tumor immune surveillance, as well as in the OVA-challenge asthma model. Wills-Karp, M., *et al.*, “Interleukin-13: central mediator of allergic asthma,” *Science* **282**(5397): 2258-2261 (1998); Grunig, G., *et al.*, “Requirement for IL-13 independently of IL-4 in experimental asthma,” *Science* **282**(5397): 2261-2263 (1998).

[0006] Gob-4 was originally cloned from mouse intestinal epithelial cells and is expressed at known cellular sites of mucin expression including stomach mucous-neck cells and intestinal goblet cells. The *Xenopus* ortholog of human Gob-4, designated XAG-2, is expressed in the cement gland, a secretory organ that expresses a mucin-like polymer allowing the *Xenopus* embryo to attach to solid supports. XAG-2 is a secreted molecule with anterior ectoderm patterning activity capable of inducing ectopic cement gland formation. This data supports the concept that human Gob-4 plays a key role in the mucus-secreting goblet cell hyperplasia that is the hallmark of asthma. Aliases for GOB-4 are hAG-2 and AGR-2.

[0007] Current therapy of asthma to treat bronchospasms and airway inflammation includes use of bronchodilators, corticosteroids, and leukotriene

inhibitors. Many of such treatments include undesired side effects and lose effectiveness after being used for a period of time. Additionally, limited agents for therapeutic intervention are available that decrease the airway remodeling process that occurs in asthmatics. Therefore, there remains a need for an increased
5 molecular understanding of asthma, coupled to identification of novel therapeutic strategies to combat this complex disease. The present invention addresses these needs.

SUMMARY OF THE INVENTION

[0008] It has been discovered that the messenger RNA (mRNA) of Gob-4
10 protein is statistically significantly increased in an animal model of asthma compared to control, non-asthmatic animals. Specifically, the mRNA encoding Gob-4 protein has been found to be elevated by either intratracheal ovalbumin challenge or direct pulmonary instillation of IL-13 and has herein been discovered as a target for asthma therapeutics. Accordingly, in one aspect of the invention,
15 methods of screening for agents for treating asthma are provided. Methods for treating asthma are also provided.

[0009] In one aspect of the invention, a method of screening for agents for treating asthma includes (a) contacting a Gob-4 protein with a test agent thought to be effective in inhibiting the activity of the Gob-4 protein; (b) determining if the
20 test agent inhibits the activity of the Gob-4 protein; and (c) classifying the test agent as an agent for treating asthma if the test agent inhibits the activity of the Gob-4 protein.

[0010] In another aspect, the invention provides a method of screening for agents for treating asthma by (a) contacting a nucleotide sequence encoding a
25 reporter gene product operably linked to a Gob-4 protein promoter with a test agent thought to be effective in inhibiting production of a Gob-4 protein; (b) determining if the test agent inhibits production of the reporter gene product; and (c) classifying the test agent as an agent for treating asthma if the test agent inhibits production of the reporter gene product.

[0011] In yet another aspect of the invention, methods for treating asthma are provided. In one embodiment, a method includes administering to a mammal in need thereof a therapeutic amount of an agent that decreases the activity of a Gob-4 protein. In a further embodiment of the invention, a method includes
5 administering to a mammal in need thereof a therapeutic amount of an agent that decreases the production of a Gob-4 protein.

DESCRIPTION OF THE FIGURES

[0012] Figure 1 is a representation of the nucleotide (cDNA) sequence of human Gob-4 protein (NCBI nucleotide database, accession no. NM_006408)
10 (SEQ ID NO:1).

[0013] Figure 2 is a representation of the amino acid sequence of human Gob-4 protein (NCBI protein database, accession no. AAP97179) (SEQ ID NO:2).

[0014] Figure 3 is a representation of the nucleotide (cDNA) sequence of mouse Gob-4 protein as disclosed by Komiyama T., *et al.*, "Cloning of the gene Gob-4, which is expressed in intestinal goblet cells in mice," *Biochim Biophys Acta*,
15 March 19, 1999; 1444(3):434-438 (SEQ ID NO:3). The coding sequence begins at residue 53 and ends at residue 580.

[0015] Figure 4 is a representation of the amino acid sequence for mouse Gob-4 protein as disclosed by Komiyama T., *et al.* (SEQ ID NO:4).

[0016] Figure 5 is a representation of the nucleotide (cDNA) sequence of rat Gob-4 protein as disclosed on the NCBI nucleotide database, identified by
20 sequence homology (accession no. XM 216691.2) (SEQ ID NO:5). The coding sequence begins at residue 54 and ends at residue 581.

[0017] Figure 6 is a representation of the amino acid sequence for rat Gob-4
25 protein (accession no. XM 216691.2) (SEQ ID NO:6).

[0018] Figure 7 (SEQ ID NO:7) is a representation of the nucleotide sequence of the promoter for human Gob-4 protein.

[0019] Figure 8 (SEQ ID NO:8) is a representation of the nucleotide sequence of the promoter for human Gob-4 protein.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The patent and scientific literature referred to herein establishes knowledge that is available to those of skill in the art. The issued U.S. patents, allowed applications, published applications (U.S. and foreign) and references, including GenBank database sequences, that are cited herein are incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference.

[0021] The invention is based upon the unexpected discovery that the mRNA of Gob-4 protein is significantly increased in an animal model of asthma compared to control, non-asthmatic animals. Specifically, the mRNA encoding Gob-4 protein is elevated by either intratracheal ovalbumin challenge or direct pulmonary instillation of IL-13. Gob-4 was originally cloned from mouse intestinal epithelial cells and is expressed at known cellular sites of mucin expression. XAG-2, the *Xenopus* ortholog of mAGR2/mGob-4, is expressed in the cement gland, a secretory organ that expresses a mucin-like polymer that allows the *Xenopus* embryo to attach to solid supports. XAG-2 is capable of inducing ectopic cement gland formation. Goblet cell metaplasia and/or hyperplasia is observed in airway epithelium following pulmonary allergic challenge. Gob-4 is considered herein to be involved in the goblet cell metaplasia and/or hyperplasia and resultant mucus hyperproduction observed in allergic asthma. Specifically, the inventors believe that Gob-4 acts as a developmental switch to help epithelial cells differentiate into goblet cells. Due to the involvement of Gob-4 proteins in the allergic response in asthma, an inhibitor of the Gob-4 proteins will be effective in treating asthma. Thus, the inventors believe that Gob-4 protein is involved in the allergic response in asthma, and, consequently, that an inhibitor of the Gob-4 protein will be effective in treating asthma. Consequently, an inhibitor of Gob-4 protein should be effective to treat asthma. "Asthma", as used herein includes, but is not limited to, atopic asthma, nonatopic asthma, allergic asthma, exercise-induced asthma, drug-induced asthma, occupational asthma and late stage asthma.

[0022] As noted above, the invention provides methods of screening for agents for treating asthma in a mammal. In one embodiment, the mammal is a human.

As used herein, "agent" includes, but is not limited to, synthetic small molecules, chemicals, nucleic acids, peptides and proteins such as hormones, antibodies and portions thereof. In one aspect, the methods include contacting a Gob-4 protein with a test agent that is thought to be effective in modulating (*e.g.*, inhibiting or increasing) the activity of Gob-4 protein. A "test agent" is a putative "agent," the modulating ability of which has not yet been confirmed. Once test agents are screened, they are classified as "agents," if they are shown to modulate protein activity or transcription. In a particular embodiment, the activity of Gob-4 protein is inhibited. The methods include: determining if the test agent modulates (*e.g.*, inhibits) the activity of the Gob-4 protein and classifying the test agent as an agent for treating asthma if the test agent modulates (*e.g.*, inhibits) the activity of the Gob-4 protein. The nucleotide and amino acid sequences of human Gob-4 protein are set forth in SEQ ID NO:1 and SEQ ID NO:2, as provided in Figures 1 and 2, respectively. The nucleotide and amino acid sequences of murine Gob-4 protein are set forth in SEQ ID NO:3 and SEQ ID NO:4, as provided in Figures 3 and 4, respectively. The nucleotide and amino acid sequences of rat Gob-4 protein are set forth in SEQ ID NO:5 and SEQ ID NO:6, as provided in Figures 5 and 6, respectively.

[0023] The discovery that Gob-4 protein is associated with inducing the symptoms and/or complications of asthma renders the sequences of Gob-4 protein useful in methods of identifying agents of the invention. Such methods include assaying potential agents for the ability to inhibit Gob-4 protein activity. Polynucleotides and polypeptides useful in these assays include not only the genes and encoded polypeptides disclosed herein, but also variants thereof that have substantially the same activity as wild-type genes and polypeptides. "Variants" as used herein, includes polynucleotides or polypeptides containing one or more deletions, insertions or substitutions, as long as the variant retains substantially the same activity of the wild-type polynucleotide or polypeptide. With regard to polypeptides, deletion variants are contemplated to include fragments lacking portions of the polypeptide not essential for biological activity, and insertion

variants are contemplated to include fusion polypeptides in which the wild-type polypeptide or fragment thereof has been fused to another polypeptide.

[0024] Accordingly, the Gob-4 protein utilized in the invention may be encoded by a nucleotide sequence that has at least about 60%, at least about 70%, at least about 80% or at least about 90% identity to the nucleotide sequence set forth in SEQ ID NO:1 (Figure 1), SEQ ID NO:3 (Figure 3), or SEQ ID NO:5 (Figure 5). Percent identity may be determined, for example, by comparing sequence information using the advanced BLAST computer program, version 2.0.8, available from the National Institutes of Health.

[0025] Additionally, the Gob-4 protein may be encoded by nucleotide sequences having substantial similarity to the nucleotide sequence set forth in SEQ ID NO:1 (Figure 1), SEQ ID NO:3 (Figure 3), or SEQ ID NO:5 (Figure 5). "Substantial similarity," as used herein means that the nucleotide sequence is sufficiently similar to a reference nucleotide sequence that it will hybridize therewith under moderately stringent conditions. This method of determining similarity is well known in the art to which the invention pertains. Examples of stringency conditions are shown in Table 1 below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

TABLE 1

Stringency Condition	Poly-nucleotide Hybrid	Hybrid Length (bp) ¹	Hybridization Temperature and Buffer ²	Wash Temperature and Buffer ²
A	DNA:DNA	> 50	65°C; 1X SSC -or- 42°C; 1X SSC, 50% formamide	65°C; 0.3X SSC
B	DNA:DNA	<50	T _B *; 1X SSC	T _B *; 1X SSC
C	DNA:RNA	> 50	67°C; 1X SSC -or- 45°C; 1X SSC, 50% formamide	67°C; 0.3X SSC
D	DNA:RNA	<50	T _D *; 1X SSC	T _D *; 1X SSC

Stringency Condition	Poly-nucleotide Hybrid	Hybrid Length (bp) ¹	Hybridization Temperature and Buffer ²	Wash Temperature and Buffer ²
E	RNA:RNA	>50	70°C; 1X SSC -or- 50°C; 1X SSC, 50% formamide	70°C; 0.3xSSC
F	RNA:RNA	<50	T _F *; 1X SSC	T _F *; 1X SSC
G	DNA:DNA	>50	65°C; 4X SSC -or- 42°C; 4X SSC, 50% formamide	65°C; 1X SSC
H	DNA:DNA	<50	T _H *; 4X SSC	T _H *; 4X SSC
I	DNA:RNA	>50	67°C; 4X SSC -or- 45°C; 4X SSC, 50% formamide	67°C; 1X SSC
J	DNA:RNA	<50	T _J *; 4X SSC	T _J *; 4X SSC
K	RNA:RNA	>50	70°C; 4X SSC -or- 50°C; 4X SSC, 50% formamide	67°C; 1X SSC
L	RNA:RNA	<50	T _L *; 2X SSC	T _L *; 2X SSC
M	DNA:DNA	>50	50°C; 4X SSC -or- 40°C; 6X SSC, 50% formamide	50°C; 2X SSC
N	DNA:DNA	<50	T _N *; 6X SSC	T _N *; 6X SSC
O	DNA:RNA	>50	55°C; 4X SSC -or- 42°C; 6X SSC, 50% formamide	55°C; 2X SSC
P	DNA:RNA	<50	T _P *; 6X SSC	T _P *; 6X SSC
Q	RNA:RNA	>50	60°C; 4X SSC -or- 45°C; 6X SSC, 50% formamide	60°C; 2X SSC
R	RNA:RNA	<50	T _R *; 4X SSC	T _R *; 4X SSC

¹The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

²SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

5 $T_B^* - T_R^*$: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C) = 2(\# \text{ of A + T bases}) + 4(\# \text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10} Na^+) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and Na^+ is the concentration of sodium ions in the hybridization buffer (Na^+ for 1xSSC = 0.165 M).

[0026] Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual", Chs. 9 & 11, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and Ausubel *et al.*, eds., Current Protocols in Molecular Biology, §§ 2.10, 6.3-6.4, John Wiley & Sons, Inc. (1995), herein incorporated by reference.

[0027] Gob-4 protein may be produced by methods known to the skilled artisan. For example, a nucleotide sequence encoding a Gob-4 protein gene may be introduced into a desired host cell. Such a nucleotide sequence may first be inserted into an appropriate recombinant expression vector.

[0028] Recombinant expression vectors may be constructed by incorporating the above-recited nucleotide sequences within a vector according to methods well known to the skilled artisan. A wide variety of vectors are known that are useful in the invention. Suitable vectors include plasmid vectors and viral vectors, including retrovirus vectors, adenovirus vectors, adeno-associated virus vectors and herpes viral vectors. The vectors may include other known genetic elements necessary or desirable for efficient expression of the nucleic acid in a specified host cell, including regulatory elements. For example, the vectors may include a promoter and any necessary enhancer sequences that cooperate with the promoter to achieve transcription of the gene. The nucleotide sequence may be operably linked to such regulatory elements.

[0029] As used herein, a nucleotide sequence is "operably linked" to another nucleotide sequence when it is placed in a functional relationship with another nucleotide sequence. For example, if a coding sequence is operably linked to a promoter sequence, this generally means that the promoter may promote transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two

protein coding regions, contiguous and in reading frame. However, since enhancers may function when separated from the promoter by several kilobases and intron sequences may be of variable lengths, some nucleotide sequences may be operably linked but not contiguous. A wide variety of methods are available for introducing the nucleotide sequence encoding a Gob-4 protein, and which may be included in a recombinant expression vector, into a host cell. Such methods are known to the art and include mechanical methods, chemical methods, lipophilic methods and electroporation. Microinjection and use of a gene gun with, for example, a gold particle substrate for the DNA to be introduced is a representative, non-limiting exemplary mechanical method. Use of calcium phosphate or DEAE-Dextran is a representative, non-limiting exemplary chemical method. Exemplary lipophilic methods include use of liposomes and other cationic agents for lipid-mediated transfection. Such methods are well known to the art.

[0030] A wide variety of host cells may be utilized in the present invention to produce the desired quantities of a Gob-4 protein. Such cells include, but are not limited to, eukaryotic and prokaryotic cells, including mammalian cells and bacterial cells known to the art.

[0031] The Gob-4 protein may be isolated and purified by techniques well known to the skilled artisan, including, but not limited to, chromatographic, electrophoretic and centrifugation techniques. Such methods are known to the art.

[0032] The Gob-4 protein is typically contacted with a test agent for a time period sufficient to inhibit the activity of the Gob-4 protein. This time period may vary depending on the nature of the inhibitor and the Gob-4 protein selected. The skilled artisan without undue experimentation may readily determine such times. An exemplary test agent is one that binds to or otherwise decreases the activity of the Gob-4 protein, although test agents that inhibit Gob-4 protein by, for example, binding to Gob-4 protein blocking interaction with receptors or proteins involved in signal transduction processes, or by some other mechanism, are also envisioned.

[0033] A wide variety of assays may be utilized to determine whether the test agent inhibits the activity of the Gob-4 protein. As the Gob-4 proteins are

involved in the differentiation of epithelial cells into goblet cells, one assay for the activity of Gob-4 is quantitation of mucus produced from the goblet cells stained with H&E, a technique known to those of skill in the art. In one embodiment, epithelial cells are contacted or otherwise incubated with an effective amount of a Gob-4 protein and the test agent. This amount is effective for stimulating differentiation of the epithelial cell cells into goblet cells and can be determined by the skilled artisan. Mucus production may then be measured and may be compared to control cells treated with the Gob-4 protein in the absence of the test agent. In addition to H&E staining for mucus production, decreases in mucus encoding mRNA expression can be measured using classical reverse transcriptase polymerase chain reaction (RT-PCR), global profiling of expressed mRNA using cDNA or oligonucleotide arrays, or quantitating the amount of mucopolysaccharides in the mucus.

[0034] A wide variety of assays are available for quantitating mucus, including use of stains to stain mucosal components and quantitation by a colorimetric assay. In one embodiment, a periodic acid Schiff technique may be used to stain mucins, glycoproteins or mucopolysaccharides present in mucus. Alternatively, mucins may be radiolabelled and analyzed by hydrophobic interaction chromatography as described in Svitacheva, N. and Davies, J.R., "Mucin biosynthesis and secretion in tracheal epithelial cells in primary culture," *Biochem. J.* 353:23-32 (2001). The mucus may be isolated by standard methods known to the art and as described, for example, in Svitacheva, N. and Davies, J.R., *supra*. Briefly, cells in culture may be harvested and the mucins may be isolated by isopycnic density-gradient centrifugation after dialyzing the samples.

[0035] Mammalian epithelial cells from a wide variety of sources may be utilized in the above-referenced screening assays involving quantitation of mucin. Nonlimiting examples of epithelial cells are epithelial cells are small airway epithelial cells or bronchial epithelial cells, which can be obtained from Clonetics (www.cambrex.com). Such cells are typically present as a cell culture. Cell culture methods are known to the skilled artisan, and described in, for example, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, John Wiley & Sons;

A.J. Shaw, ed., *Epithelial Cell Culture: A Practice Approach* (Practical Approach Series), IRL press, 1996; R.I. Freshney, and M.G. Freshney, eds., *Culture of Epithelial Cells*, 2nd Edition, John Wiley & Sons, 2002; and C. Wise, ed., *Epithelial Cell Culture Protocols* (Methods in Molecular Biology, v. 188), Humana Press, 2002. In addition, epithelial cell lines may be used in screening assays involving the quantitation of mucin. Alternatively, in yet another embodiment, the amount of epithelial cell-derived goblet cells may be quantitated. That is, the number of goblet cells formed from differentiation of epithelial cells by the action of Gob-4 may be quantitated. Methods of quantitation include, without limitation, staining for mucus production using periodic acid-Schiff (PAS)/alcian blue and microscopic examination for increased numbers of goblet epithelial cells or an increase in secretory granules within specific cells. Kondo *et al.*, "Interleukin-13 Induces Goblet Cell Differentiation in Primary Cell Culture from Guinea Pig Tracheal Epithelium" Am. J. Respir. Cell Mol. Biol. Nov. 2002 27(5):536-541.

[0036] A wide variety of test agents may be tested in the screening methods of the present invention. For example, small molecule compounds, known in the art, chemicals, nucleic acids, peptides and proteins such as hormones, antibodies, and portions thereof, may act as test agents. In one nonlimiting example, the three-dimensional structure of the active site of Gob-4 protein is determined by crystallizing the complex formed by the protein and a known inhibitor. Rational drug design is then be used to identify new test agents by making alterations in the structure of a known inhibitor or by designing small molecule compounds that bind to the active site of the protein.

[0037] The invention also provides a method of screening for agents for treating asthma in a mammal. In one embodiment, the invention includes contacting a nucleotide sequence encoding a reporter gene product operably linked to a Gob-4 protein promoter, with a test agent thought to be effective in inhibiting production of Gob-4 protein; determining if the test agent inhibits production of the reporter gene product; and classifying the test agent as an agent for treating asthma if the test agent inhibits production of the reporter gene product. In one

embodiment, the mammal is a human. "Asthma", as used herein includes, but is not limited to, atopic asthma, nonatopic asthma, allergic asthma, exercise-induced asthma, drug-induced asthma, occupational asthma and late stage asthma.

[0038] The Gob-4 protein promoter preferably includes a nucleotide sequence set forth in SEQ ID NO:7 or SEQ ID NO:8, as set forth in Figures 7 and 8, respectively. SEQ ID NO:7 and SEQ ID NO:8 each depict the sequence located two kilobases upstream of the mRNA initiation codon. These sequences were identified electronically using publicly available sequence data known to those of skill in the art. Nucleotide sequences having at least about 50%, at least about 70%, at least about 80% and at least about 90% identity to such sequences and that function as promoter, for example, to direct expression of a gene encoding a Gob-4 protein described herein, are also encompassed in the invention.

[0039] The nucleotide sequence of the Gob-4 protein promoter is determined by art-recognized methods. One nonlimiting example of such a method is to screen a genomic library (*e.g.*, a YAC human genomic library) for the promoter sequence of interest using SEQ ID NO:1 (Figure 1), SEQ ID NO:3 (Figure 3), or SEQ ID NO:5 (Figure 5) as a probe. Another nonlimiting example of a method to determine the appropriate promoter sequence is to perform a Southern blot of the human genomic DNA by probing electrophoretically resolved human genomic DNA with a probe (*e.g.*, a probe comprising SEQ ID NO:1 or a portion thereof) and then determining where the cDNA probe (*e.g.*, SEQ ID NO:1) hybridizes. Upon determining the band to which the probe (*e.g.*, SEQ ID NO:1) hybridizes, the band can be isolated (*e.g.*, cut out of the gel) and subjected to sequence analysis. This allows detection of the nucleotide fragment 5' of nucleotides 104-106 (*i.e.*, the ATG site) of SEQ ID NO:1. The nucleotide fragment may be between approximately 500 to 1000 units in length. The promoter sequence for murine Gob-4 protein set forth in SEQ ID NO:3 (Figure 3) or the promoter sequence for rat Gob-4 protein set forth in SEQ ID NO:5 (Figure 5) may be determined by these methods as well. Nucleotide sequences having at least about 70%, at least about 80% and at least about 90% identity to such sequences and that

function as promoter, for example, to direct expression of a gene encoding a Gob-4 protein described herein, are also encompassed in the invention.

[0040] A wide variety of reporter genes may be operably linked to the Gob-4 protein promoter described above. Such genes may encode, for example,
5 luciferase, β -galactosidase, chloramphenicol acetyltransferase, β -glucuronidase, alkaline phosphatase, and green fluorescent protein, or other reporter gene products known to the art.

[0041] In an embodiment of the invention, the nucleotide sequence encoding a reporter gene that is operably linked to a Gob-4 protein promoter is introduced into
10 a host cell. Such a nucleotide sequence may first be inserted into an appropriate recombinant expression vector as previously described herein.

[0042] The vectors in this aspect of the invention may include other known genetic elements necessary or desirable for efficient expression of the nucleic acid sequence from the Gob-4 protein promoter in a specified mammalian cell,
15 including regulatory elements. For example, the vectors may include any necessary enhancer sequences that cooperate with the promoter in vivo, for example, to achieve in vivo transcription of the reporter gene. The methods of introducing the nucleotide sequence into a host cell are identical to that previously described for producing the Gob-4 protein.

[0043] A wide variety of host cells may be utilized in the methods of screening in the present invention. Exemplary host cells include, for example,
20 Chinese hamster ovary, E. coli, COS and Bacillus.

[0044] Alternatively, the nucleotide sequence encoding all or a portion of the Gob-4 protein gene may be utilized in the vector for the screening methods
25 described herein. In such a case, Gob-4 protein may be isolated and purified by techniques well known to the skilled artisan, including chromatographic, electrophoretic and centrifugation techniques, as previously described herein. Additionally, Gob-4 protein may be quantified by methods known to the art.

[0045] After contacting a nucleotide sequence encoding a reporter gene, or a Gob-4 protein gene, operably linked to a Gob-4 protein promoter with a test agent
30

thought to be effective in inhibiting production of a Gob-4 protein, it is determined if the test agent inhibits production of the reporter gene product. This endpoint may be determined by quantifying either the amount or activity of the reporter gene product. The method of quantification will depend on the reporter gene that
5 is used, but may involve use of an enzyme-linked immunosorbent assay with antibodies to the reporter gene product. Additionally, the assay may measure chemiluminescence, fluorescence or radioactive decay, or other methods known in the art. Assays for determining the activity or amount of the reporter gene products described herein are known to the art. If the test agent inhibits
10 production of the reporter gene product, it is classified as an agent for treating asthma.

[0046] The invention also provides methods for treating asthma. "Treatment", "treating" or "treated" as used herein, means preventing, reducing or eliminating at least one symptom or complication of asthma. Exemplary symptoms and/or
15 complications of asthma include, but are not limited to, AHR, mucus hyperproduction, elevated serum IgE levels, elevated airway eosinophilia and airway remodeling. These methods include administering to a mammal, *e.g.*, a human, in need thereof a therapeutic amount of an agent that decreases the production or activity of a Gob-4 protein. A "therapeutic amount" represents an
20 amount of an agent that is capable of inhibiting or decreasing the production of Gob-4 protein and causes a clinically significant response. The clinical response includes an improvement in the condition treated or in the prevention of the condition. The particular dose of the agent administered according to this invention will, of course, be determined by the particular circumstances
25 surrounding the case, including the agent administered, the particular asthma being treated and similar conditions.

[0047] Agents that decrease the activity of a Gob-4 protein include those agents discovered in the screening assays described herein. Additional agents, or inhibitors, are well known in the art and include, for example, IL-13R α 2 and
30 antibodies against a Gob-4 protein. See, *e.g.*, Houston, *et al.*, PNAS 99(14): 9127-9132 (2002). An antibody against the Gob-4 protein as used herein may be,

without limitation, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a genetically engineered antibody, a bispecific antibody, antibody fragments (including but not limited to "Fv," "F(ab')₂," "F(ab)," and "Dab") and single chains representing the reactive portion of the antibody. Methods for production of each of the above antibody forms are well known to the art.

[0048] In another aspect, the invention provides methods for treating asthma that includes administering to a mammal in need thereof a therapeutic amount of an agent that decreases the production of a Gob-4 protein. In one embodiment, the mammal is a human. In another embodiment, the agent is administered in a pharmaceutically acceptable carrier.

[0049] In one embodiment, the agent that decreases the production of the Gob-4 protein is a nucleic acid. Exemplary nucleic acids include, but are not limited to, a deoxyribonucleic acid or a ribonucleic acid. In one embodiment, the ribonucleic acid has a nucleotide sequence that is complementary to a portion of the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, as set forth in Figures 1, 3 and 5, encoding acidic mammalian protein.

[0050] In another embodiment, RNA interference may be used as an inhibitor of Gob-4 protein. RNA interference relates to sequence-specific, posttranscriptional gene silencing brought about by double-stranded RNA that is homologous to the silenced gene target. Methods for inhibiting production of a protein utilizing small interfering RNAs are well known to the art, and disclosed in, for example, PCT International Application Numbers WO 01/75164; WO 00/63364; WO 01/92513; WO 00/44895; and WO 99/32619.

[0051] The agents may be administered by a wide variety of routes. Exemplary routes of administration include oral, parenteral, transdermal, and pulmonary administration. For example, the agents may be administered intranasally, intramuscularly, subcutaneously, intraperitoneally, intravaginally and any combination thereof. For pulmonary administration nebulizers, inhalers or aerosol dispensers may be used to deliver the therapeutic agent in an appropriate

formulation (*i.e.*, with an aerolizing agent). In addition, the agents may be administered alone or in combination with other agents or known drugs. In combination, agents may be administered simultaneously or each agent may be administered at different times. When combined with one or more known asthma drugs, agents and drugs may be administered simultaneously or the agent can be administered before or after the drug(s).

[0052] In one embodiment, the agents are administered in a pharmaceutically acceptable carrier. Any suitable carrier known in the art may be used. Carriers that efficiently solubilize the agents are preferred. Carriers include, but are not limited to a solid, liquid or a mixture of a solid and a liquid. The carriers may take the form of capsules, tablets, pills, powders, lozenges, suspensions, emulsions or syrups. The carriers may include substances that act as flavoring agents, lubricants, solubilizers, suspending agents, binders, stabilizers, tablet disintegrating agents and encapsulating materials.

[0053] Tablets for systemic oral administration may include excipients, as known in the art, such as calcium carbonate, sodium carbonate, sugars (*e.g.*, lactose, sucrose, mannitol, sorbitol), celluloses (*e.g.*, methyl cellulose, sodium carboxymethyl cellulose), gums (*e.g.*, arabic, tragacanth), together with disintegrating agents, such as maize, starch or alginic acid, binding agents, such as gelatin, collagen or acacia and lubricating agents, such as magnesium stearate, stearic acid or talc.

[0054] In powders, the carrier is a finely divided solid, which is mixed with an effective amount of a finely divided agent.

[0055] In solutions, suspensions or syrups, an effective amount of the agent is dissolved or suspended in a carrier such as sterile water or an organic solvent, such as aqueous propylene glycol. Other compositions can be made by dispersing the inhibitor in an aqueous starch or sodium carboxymethyl cellulose solution or a suitable oil known to the art.

[0056] The agents are administered in a therapeutic amount. Such an amount is effective in treating asthma. This amount may vary, depending on the activity

of the agent utilized, the nature of the asthma and the health of the patient. The term "therapeutically effective amount" is used to denote treatments at dosages effective to achieve the therapeutic result sought. Furthermore, a skilled practitioner will appreciate that the therapeutically effective amount of the agent may be lowered or increased by fine tuning and/or by administering more than one agent, or by administering an agent with an anti-asthmatic compound (*e.g.*, corticosteroid). As illustrated in the following examples, therapeutically effective amounts may be easily determined, for example, empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of beneficial effect. (*i.e.*, reduction in asthmatic symptoms following exposure to antigen).

[0057] When the agents are combined with a carrier, they may be present in an amount of about 1 weight percent to about 99 weight percent, the remainder being composed of the pharmaceutically acceptable carrier.

[0058] Reference will now be made to specific examples illustrating the invention. It is to be understood that the examples are provided to illustrate preferred embodiments and that no limitation to the scope of the invention is intended thereby.

EXAMPLE 1

Gene Expression Changes in Mouse Lung Associated with Allergic Reaction

[0059] To identify the gene expression changes induced by intratracheal OVA-challenge Balb/C mice (Jackson Laboratories (Bar Harbor, ME)) were immunized by an intraperitoneal (i.p.) injection of 10 μ g of ovalbumin (OVA) (Sigma, St. Louis, MO) in 200 μ l of PBS on day 0. On days 14 and 25, mice were anesthetized with a mixture of ketamine and xylazine (45 and 8 mg/kg respectively) and challenged intratracheally with 50 μ l of a 1.5% solution of OVA or an equivalent volume of PBS. To identify changes in mRNA concentration dependent on IL-13 mediated signal transduction, two of the OVA-challenged mice were treated with three intraperitoneal injections of the soluble IL-13 receptor fusion protein, sIL-13R α 2-Fc, prior to and during the course of the allergic challenge. As control for

the Fc-moiety of the receptor fusion protein, two of the OVA-challenged mice were similarly treated with intraperitoneal administration of hIgG. A second set of six control mice were similarly sensitized to OVA without subsequent challenge and treated on an identical time course with intratracheal administration of PBS buffer, either alone (n=2) or with intraperitoneal injection of hIgG (n=2) or sIL-13R α 2-Fc (n=2). Lung tissue for the OVA-challenged and buffer-alone control mice was harvested at 78hr following the second pulmonary antigen challenge (day 28).

[0060] Snap frozen mouse lung tissue was pulverized using liquid nitrogen chilled mortar and pestle, suspended in 6 ml 4M guanidinium isothiocyanate/0.7% 2-mercaptoethanol (GTC/ME) and pulse sonicated for 2 minutes. The tissue suspension was extracted twice with acid equilibrated phenol (Promega Total RNA Kit) and nucleic acid precipitated with an equal volume of isopropanol. The pellet was resuspended in 0.8 ml GTC/ME, reextracted twice with an equal volume of acid phenol and once with chloroform. RNA was ethanol-precipitated, suspended in DEPC treated H₂O and quantified by OD280.

[0061] cDNA was synthesized from 10 μ g of total RNA using the Superscript Kit (BRL) with modifications described in Byrne, *et al.*, "Preparation of mRNA for expression monitoring," *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. (New York 2000). First strand synthesis was carried out at 50°C to prevent mispriming from ribosomal RNA and utilized a T7 RNA polymerase promoter containing poly-T primer (T7T24) for subsequent *in vitro* antisense RNA (cRNA) amplification and biotin labeling. cDNA was purified using BioMag Carboxyterminated beads (Polysciences) according to manufacturer's instructions, and eluted in 48 μ l of 10mM NaAcetate pH 7.8.

[0062] *In vitro* T7 polymerase driven transcription reactions for synthesis and biotin labeling of antisense cRNA, Qiagen RNeasy spin column purification and cRNA fragmentation were carried out. GeneChip® hybridization mixtures contained 10 μ g fragmented cRNA, 0.5 mg/ml acetylated BSA, 0.1 mg/ml herring sperm DNA, in 1X MES buffer in a total volume of 200 μ l as per manufacturer's instructions. Reaction mixtures were hybridized for 18 hours at 45°C to

Affymetrix Mu11KsubA and Mu11KsubB oligonucleotide arrays. The hybridization mixtures were removed and the arrays were washed and stained with Streptavidin R-phycoerythrin (Molecular Probes) using the GeneChip® Fluidics Station 400 and scanned with a Hewlett Packard GeneArray Scanner following manufacturer's instructions. Fluorescent data was collected and converted to gene specific difference averages using MicroArray Suite 4.0 software.

[0063] An eleven-member standard curve was prepared by spiking gene fragments derived from cloned bacterial and bacteriophage sequences into each hybridization mixture at concentrations ranging from 0.5pM to 150pM. These standards represented RNA frequencies of approximately 3.3 to 1000 parts per million (ppm) assuming an average transcript size of 2kb. The biotinylated standard curve fragments were synthesized by T7 polymerase driven IVT reactions from plasmid-based templates. The spiked biotinylated RNA fragments served both as an internal standard to assess chip sensitivity and as standard curve to convert measured fluorescent difference averages from individual genes into RNA frequencies in ppm. Average fluorescence difference between perfect match and single mismatch probe sets containing gene-specific oligonucleotides were used to determine frequency values with respect to spiked standard curve. In addition, a second set of algorithms based primarily on the fraction of individual positive or negative responding probe pairs, was used to assess the absolute presence or absence of the gene product. The sensitivity of the individual microarray chip was set at one-half the minimum concentration at which 2 or any 3 adjacent standard curve spike-in templates were called present. The standard curve linear regression was forced through zero and the minimum reported gene frequency was set to the sensitivity of the individual GeneChip®.

[0064] Multiple independent replicas for each of the treatment or control experimental conditions were measured and the expression data subjected to routine statistical analysis in an effort to remove false positives. Frequency values determined from individual measurements for a given experimental set were initially compared. Average values for treatment and control animals were compared to obtain average fold change (AFC). Two tailed Student t-tests were

calculated using either unequal covariance with raw frequency values or equal covariance with log-transformed frequency values.

[0065] The overall gene expression measured for each of the three treatment groups used to identify allergen-challenge induced gene expression was well
 5 balanced with respect to mRNA integrity, number of genes called present and total mRNA frequency computed across the various control and treatment files (data not shown).

[0066] The gene expression profile measured for control mice treated with PBS was not significantly altered by intraperitoneal co-administration of human
 10 IgG or sIL-13R α 2-Fc, and thus frequency values from the six control mice were combined as a single set in the calculation of average untreated baseline expression values. Similarly, the four OVA-challenged mice treated either with intraperitoneal co-administration of buffer or hIgG were combined as a single set in calculation of average frequency values for pulmonary allergen-challenged
 15 mRNA frequency. The data are shown on Table 2 below.

**Table 2. mRNA frequency values for Gob-4 protein
 following challenge**

Sample	mRNA Frequency (ppm)
PBS Control	6.5
OVA and sILR α 2-Fc	7.0
OVA	40.8

[0067] The data demonstrate that Gob-4 protein mRNA is specifically induced
 20 by direct pulmonary intratracheal administration of IL-13 or ovalbumin-induced allergic challenge. Additionally, these data show that inhibition of IL-13 activating using the soluble receptor antagonist (sIL-13R α 2-Fc) completely inhibits the expression of Gob-4 protein by ovalbumin challenge. Physiological studies utilizing the sIL-13R α 2-Fc antagonist have previously shown IL-13
 25 activity is essential to asthma disease pathology, including epithelial mucus production and AHR. Thus, Gob-4 protein is identified as an IL-13 responsive

gene downstream of the ovalbumin allergic challenge, which identifies it as a therapeutic agent in the target of asthma.

[0068] These data confirm that intraperitoneal administration of the soluble IL-13 antagonist was able to completely inhibit the induction of OVA allergic pulmonary Gob-4 protein. Physiological studies utilizing the sIL-13R α 2-Fc antagonist has previously shown to be IL-13 activity essential for asthma disease pathology including epithelial mucus production and AHR. Wills-Karp, M, *et al.*, *Science* 282(5397):2258-61 (1998). Thus, these data demonstrate that therapeutic intervention resulting in the inhibition of Gob-4 protein is associated with a decrease of asthma related symptoms.

EXAMPLE 2

Gene Expression Changes in Mouse Lung Induced by mIL-13 Lung Instillation

[0069] To identify IL-13 mediated changes in pulmonary gene expression, six Balb/C mice (Jackson Laboratories, Bar Harbor, ME) were treated with multiple 5 μ g dose (0, 24hr, and 48hr) lung instillation of recombinant mIL-13. A second set of control Balb/C mice (n=4) were instilled with buffer alone on an identical schedule. Additionally, a set of Stat6^{-/-} null mice were treated identically with multiple doses mIL13 (n=4) or PBS buffer (n=5) lung instillation prior to harvesting of all lungs at 78hr for expression profiling. Stat6^{-/-} is an additional control; it is a key intermediate in IL-13 signaling pathway, critical for mucus production and AHR; the absence of this IL-13 signaling transducer ameliorates asthmatic symptoms. The overall gene expression for each of the three treatment groups used to identify allergen-challenge induced gene expression was well-balanced with respect to mRNA integrity, number of genes called present, and total mRNA frequency computed across the various control and treatment files. The data are shown in Table 3.

Table 3

Animal	Treatment	Gob-4 protein mRNA Frequency (ppm)
Balb/C	PBS	9.8
	rIL-13	108.8
Stat6 ^{-/-}	PBS	7.8
	rIL-13	11.8

[0070] These data confirm that increases in Gob-4 mRNA concentration are mediated by mIL-13 lung instillation.

5

EXAMPLE 3**Prophetic example of screening assay for modulator of Gob-4 protein activity**

[0071] Human Gob-4 protein (hGob-4 protein) is cloned into bacterial expression vector, transformed into E. coli or COS cells and the protein purified from bacterial or mammalian cultures by column chromatography utilizing standard molecular biology and biochemistry methods. Primary epithelial cells or epithelial cell lines are then treated with the Gob-4 protein. After contact with the protein, the cells or cell lines are assayed for an increase in goblet cells or goblet cell-expressed mucin. Test agents will be screened by their ability to modulate (e.g., inhibit) the mucus production as determined by staining of the epithelial cells or by microscopic observation of increased secretory granules.

10
15**EXAMPLE 4****Prophetic example of screening assay for inhibitor of Gob-4 protein production involving Gob-4 protein promoter**

[0072] A Gob-4 protein promoter is linked to a reporter gene, for example, a luciferase. Activation of the reporter gene is demonstrated by inducing with IL-13, indicating transcriptional specificity. Test agents are screened to identify those that block the IL-13 induced reporter gene activity.

20

EXAMPLE 5**Treating Asthma with Gob-4 protein Inhibitor**

[0073] A therapeutically effective amount of a known Gob-4 protein inhibitor is administered to a subject diagnosed with asthma. A control group also
5 exhibiting asthmatic symptoms will be a placebo control. Administration may be by a single treatment or treatment over a course of days. Subjects are evaluated for asthma-related symptoms, such as AHR, forced expiratory volume and mucus production, following pulmonary antigen challenge. Effective treatment is determined by a reduction in asthma-related symptoms compared to the control
10 group.

[0074] While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes and modifications that come
15 within the spirit of the invention are desired to be protected. In addition, all references cited herein are indicative of the level of skill in the art and are hereby incorporated by reference in their entirety.

CLAIMS

What is claimed is:

1. A method of screening for agents for treating asthma in a human, comprising:

- 5 (a) contacting a Gob-4 protein with a test agent thought to be effective in inhibiting the activity of said Gob-4 protein;
- (b) determining if said test agent inhibits the activity of said Gob-4 protein, wherein determining if said test agent inhibits the activity of said mammalian Gob-4 protein comprises quantitating the amount of mucus produced by staining said mucus with a periodic-acid Schiff stain; and
- 10 (c) classifying said test agent as an agent for treating asthma if said test agent inhibits the activity of said Gob-4 protein.

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1  ccgcatccta gccgccgact cacacaagggc aggtgggtga ggaaatccag agttgccatg
61 gagaaaaattc cagtgtcagc attcttgctc cttgtggccc tctcctacac tctggccaga
121 gataccacag tcaaacctgg agccaaaaag gacacaaaagg actctcgacc caaactgccc
181 cagaccctct ccagaggttg gggtgaccaa ctcatctgga ctgagacata tgaagaagct
241 ctatataaat ccaagacaag caacaaaccc ttgatgatta ttcatcactt ggatgagtgc
301 ccacacagtc aagctttaaa gaaagtgttt gctgaaaata aagaaatcca gaaattggca
361 gagcagtttg tctcctcaa tctggtttat gaaacaactg acaaacacct ttctcctgat
421 ggccagtatg tccccaggat tatgtttgtt gacccatctc tgacagttag agccgatatc
481 actggaagat attcaaatcg tctctatgct tacgaacctg cagatacagc tctgttgctt
541 gacaacatga agaaagctct caagttagctg aagactgaat tgtaaagaaa aaaaatctcc
601 aagcccttct gtctgtcagg ccttgagact tgaaaccaga agaagtgtga gaagactggc
661 tagtgtggaa gcatagtga cactgtgatt aggttatggt ttaatgttac aacaactatt
721 ttttaagaaa aacaagtttt agaaatttgg tttcaagtgt acatgtgtga aaacaatatt
781 gtatactacc atagttagcc atgattttct aaaaaaaaaa ataaatgttt tgggggtgtt
841 ctgttttctc caacttggtc tttcacagtg gtctgtttac caaataggat taaacacaca
901 caaatgtctc aaggaaagga caagacaaaa ccaaaactag ttcaaatgat gaagaccaa
961 gaccaagtta tcatctcacc acaccacagg ttctcactag atgactgtaa gtagacacga
1021 gcttaatcaa cagaagtatc aagccatgtg ctttagcata aaagaatatt tagaaaaaca
1081 tcccaagaaa atcacatcac tacctagagt caactctggc caggaactct aaggtacaca
1141 ctttcattta gtaattaaat tttagtcaga ttttgcccaa cctaagtctc tcagggaaag
1201 cctctggcaa gtagctttct ccttcagagg tctaatttag tagaaaggtc atccaaagaa
1261 catctgcact cctgaacaca ccctgaagaa atcctgggaa ttgacctgt aatcgatttg
1321 tctgtcaagg tcctaaagta ctggagtga ataaattcag ccaacatgtg actaattgga
1381 agaagagcaa aggggtggtga cgtgttgatg aggcagatgg agatcagagg ttactagggc
1441 ttaggaaacg tgaaaggctg tggcatcagg gtaggggagc attctgccta acagaaatta
1501 gaattgtgtg ttaatgtctt cactctatac ttaatctcac attcattaat atatggaatt
1561 cctctactgc ccagccctc ctgattttct tggcccctgg actatggtgc tgtatataat
1621 gctttgcagt atctgttgct tgtcttgatt aacttttttg gataaacct ttttgaaca
1681 gaaaaaaaaa aaaaaaaaaa a
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FIG. 1

1 MEKIPVSAFLLLVALSYTLARDTTVKPGAKKDTKDSRPKL
41 PQTLSRGWGDQLIWTQTYEEALYKSKTSNKPLMI IHHLDE
81 CPHSQALKKKVFAENKEIQKLAEQFVLLNLVYETTDKHLSP
121 DGQYVPRIMFVDPSTLTVRADITGRYSNRLYAYEPADTALL
161 LDNMKKALKLLKTEL

FIG. 2

1 ggcaaccctt gcggctcaca caaagcagga ggggtgggaag cccagatttg ccatggagaa
61 attttcagtg tctgcaatcc tgcttcttgt ggccatttct ggtaccttgg ccaaagacac
121 cacagtcaaa tctggagcca aaaaggaccc aaaggactct cggcccaaac tacctcagac
181 actctccaga ggttggggcg atcagctcat ctggactcag acatacgaag aagctttata
241 cagatccaag acaagcaaca gacccttgat ggtcattcat cacttggacg aatgcccaca
301 cagtcaagcc ttaaagaaaag tgtttgctga acataaagaa atccagaaat tggcagagca
361 gtttggttctc ctcaacctgg tctatgaaac aaccgaacaag cacctttctc ctgatggcca
421 gtacgtcccc agaatttgtgt ttgtagaccc atccctgacg gtgagggcag acatcactgg
481 acgatactca aaccggctct acgcttatga accttctgac acagctttgt tgtacgacaa
541 catgaagaaa gctctcaagc tgctaaagac agaattgtag agctaactgc gcaccgggtc
601 aggagaccag aaggcagaag cactgtggac ttgcagatta cagtacagtt taatgttaca
661 acagatatat tttttaaaca cccacaggtg gggaaacaat attattatct actacagtga
721 agcatgattt tctagaaaat aaagtcttgt gagaactcca aaaaaaaaaa aaaaaaaaaa

FIG. 3

MEKFSVSAILLLVAISGTLAKDTTVKSGAKKDPKDSRPKLPQTLSRGWGDQLIWTQTYEEALYRS
KTSNRPLMVIHHLDECPHSQALKKVFAEHKEIQKLAEQFVLLNLVYETTDKHLSPDGQYVPRIVF
VDPSLTVRADITGRYSNRLYAYEPSDTALLYDNMKKALKLLKTEL

FIG. 4

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1  cggcaaccct  tgcggctcac  acaaagcagg  agggaggaga  gctcagattt  gccatggaga
61  aathttcagt  ctcggaatc  ctgcttcttg  tggccatctc  tggtagctctg  gccaaagaca
121 ccacagtcaa  atctggatcc  aaaaaggacc  caaaggactc  tcgacccaaa  ctaccccaga
181 ccctgtccag  aggttgggga  gatcagctca  tctggactca  gacttacgaa  gaagccttat
241 acaaatccaa  gacaagcaac  agacccttga  tggtcattca  tcacttggac  gaatgcccg
301 acagtcaagc  tttaaagaaa  gtgtttgctg  aaaataagga  gatccagaaa  ttggcagagc
361 agtttgttct  cctcaacttg  atctatgaaa  caactgacaa  gcacctttct  cctgatggcc
421 agtacgtccc  cagaattgtg  tttgtggacc  ctccctgac  ggtgagggca  gacatcaccc
481 gaagatactc  aaaccgtctc  tacgcttacg  aaccttctga  cacagctctg  ctgcacgaca
541 acatgaagaa  agctctcaag  ttgctgaaga  cagagttgta  gagtcaactg  tacagtgcct
601 caggagccgg  gaaggcagaa  gcactgtgga  cctgccgatg  acattacagt  ttaatgttac
661 aacaaatgta  ttttttaaac  acccacgtgt  ggggaaacaa  tattattatc  tactacagac
721 acatgatttt  ctagaaaata  aagtcctgtg  agaactcc
```

FIG. 5

MEKFSVSAILLLVAISGTLAKDTTVKSGSKDPKDSRPKLPQTLSRGWDQLIWTQTYEEALYKS
KTSNRPLMVIHHLDECPHSQALKKVFAENKEIQKLAEQFVLLNLIYETTDKHLSPDGQYVPRIVF
VDPSLTVRADITGRYSNRLYAYEPSDTALLHDNMKKALKLLKTEL

FIG. 6

1 AACCTAGTT ACCTCACACC AAGACAGATA TGCCAAAGAT TCCACAGCCT
51 CAATAGCATG TGTAGGATAT CTGCTAATAA TTACCTCCTC CTTGCCATCC
101 GTCAGCCACT ATGACAAACT CTGGGTTTTT CCTGACATGA GATTAGGCAC
151 ATGAGTATAG AATAATTATA TCACTATAAT TAACTGTAAC AAATCAAAGA
201 CTTTTTTTTT TAAGTTCCGG AGTATGTGTG TAGGATGTGC AGGTTTGTTT
251 CATCAGTAAA CGTGTGCCAT GGTGGTTTGC TGCCTGATC AACCCAACAA
301 CTAGGTCTTA AGCCAGCCTG CATTAGCTAC TTTTATCAAA TGTTATGGGC
351 TGAATTGTGT CCCCCCAAA AATTCATATG TTGAAGTCTT AATCCCCAGG
401 ACTTCAGAAT AGGATCTTTA CAGAGGTAAT TAAGTTAAAG TAGGTCATTA
451 GGCAGGACCC AAATACAATA TGACTGGTGT CCTTATAAGA AAAGGAAAAA
501 AATGACACAG ACAGGTACAG AGGGAAAAAC CATGTGGCAA TACAGGGAAA
551 AGTCATTTAA TATTCAAAAT GGTCCCATAT GTTAATATTA TCCCCATATT
601 ATAGATGGAG AACTGAAGT TTTGGGGATG TTAAATGAGA TCTCAGATCA
651 TCCTATGAGC AAGCACCAGG ATGCAGGATT CAGATGGGAA TCTCGTGA
701 CCAAATCCCA TCCACTTGTT ACTTTCAGTG GATAAGGGAC TGAAGGACTT
751 TGGTCCCAAC TCTGCCCTAA ACTAGTTGTG AGACCTTCAA AAAGTTATGA
801 ATTTTTTGCC ATCTTCATTT ATTCATCTGT AAAATGAAAG ACTGGAATTG
851 AATATTACAA GGGTCTATCT AAGGGCCTGC TAGTTTAAAG AATTTTGCTC
901 AAATCATCGT TTTCAAACCT CTGAAGAAAT TACTTCTATA AATTCATTAG
951 AATTGAAAGG AAATTCAGTA TTTGGAGAAT CACGATTTTG CCCACAGAAT
1001 TCAAGGATTT ATTGGAAAAA TATACATACT TGCAAATGTT TTTGAAATAT
1051 TATGACCTTA ACTCATTTTA AAAAGTCATT TATATAGGGC TTGCATCCCA
1101 TTCATTAACT TTCTGTTGTT AACATTTTCT TCATTCTGAG CTTTAAAGA
1151 CTGCACACAA CTTTCATGAAC AAAATACAGG ATTAAAATTT TCTGACAGAA
1201 AATTTAAATT CCAGTTTTAA AATCTTCAGG GAGTAATTAA ATGGTCTTGA
1251 GGGGAAAAAA AACTTGGTG CAGACCTTAG TTTTAGGTC TGAGAAAATG

1301 GAGTAAATGG CTTCTGCTT GCGTGGCAGG AAAGTTTGCC TTAAATAAG
1351 AGATTATCTG TGAAATACCT TTGAACTCTG TGGAGGGAAG TTGCTGCATA
1401 CATTCAATGG CAAGGCATTT ATTACAAGCT CACGATATTA GGCTGTTTTT
1451 TTTTTTTTTT TTGCCAATAC TTCCTCAGTT TTGAAAAATT ACGTGGGTTA
1501 CTTGATTGTG ATTTTTTTTC ATACCTGTAG AAGTTAGGGT GCATTGTTTT
1551 GACAGGAGCA GGAAGTATT GTAGAAAATA ATTTTATCA TAATGGAGTA
1601 TGGCAGGTTA TATGACTGCG AGGATCAGAA TTGTGAATCA TCTCTGTGT
1651 GTCTTCAAGT AAATAAAGGC AATCTGCCCA CGGAGCAGAA AAAAAATCTA
1701 CAACTACAA ACTCTGTCCA ATCATGTAAA GACAAATCAG CCTTCAGGCA
1751 AATCAAATGT CTTCAATCAA AGTCTACCTG GATTGCGCAC TCTGCCCATC
1801 GTTTCAAAC CTCTTAACAA TACGTTTCAC AAATAGTTAA AAACATGCAT
1851 ACTGAAAAGC ATACTTTTGC AATGTTATTT TTAAAAACAA GGAATCTTT
1901 AACCAGGGA AGATAATCAC TTGGGGAAAG GAAGGTCGT TTCTGAGTTA
1951 GCAACAAGTA AATGCAGCAC TGGTGGGTGG GATTGAGGTG TGCCCTGGTG
2001 CATAAATAGA GACTCAGCTG TGCTGGCACA CTCAGAAGCT TGGACCGCAT

FIG. 7

1 AAAGGTCTAG AAAGAAACCT TTAAATGAG TGAACTTTAC CATACTAGA
51 AATGCTGTGG GCTAGTGACT CTTGAAATAA CTCCATTTGC TTATGCTTCT
101 AAAAGGTCTA CAGAGACCAT TTTTTTAAAA GATGATTGAT TAAAAAAAC
151 TGATTTGAGG TAAAAACCTT AACTAGAATT GCTCTCACAT ATCTAAATAT
201 CACTATTTAG CCTTTAGTTC TATTCAAACC ATTATTTTAC AGATTAGAAA
251 CACCAAACAA ACGATTAAGC AAACAAAAT AGAACAGTCA ATAGTTTTCT
301 AAAGGCCCTA CAATTAGTTG AGGGCAGAGC TAGGAGGAAA GCCAGGGCTC
351 TTCTACTCCA CTATCTTAGG CATTGGGAAA TGGGTGGGAT TTCGGGTCAA
401 TTACAGTCAG CATCCTGCTT CCACACTCTG GATGATGATA TCAGAGGTGA
451 CACTGAACAC CCTGAAACTT TAGTTTCCAC GCCTGTAACA GAGTTCCATG
501 CAACAGTTCA GAGCGACATA GTCGTGAACA TAGAGTGAAC TGAGGAAGAG
551 GAAGAGGCTT GGGATGAACG TAGGGTCCCT GCTTCCACAG GAACAGGACA
601 GCCTGGGAGG CTGAAGCATC GGCATTAC CTTGCTCAA TCCTGGAGGC
651 TCCACACAGA CCATTGATGT GTCAGCAGCG TTAGGTTCTT CTCTTCTTGG
701 CCTGTAGATG AAGTCATTAT GTGCCTGTGT CTCTGACCTA AGTTTCTTTC
751 CTATGAGAAT AACAGTCATA TTAGATTAGA ACCCAGTCTA ATGACCTATT
801 TCACTTACTT TAAATTTCTT ATTCATTTAT TTCAATTACT TTCATTTTAT
851 TTACTIONT TGGTACTTAG AATCAAATTC AGAGCCTTGC ACATACTTAA
901 CAAATGCTTA ATCTCTCTTT AAGACCCTCT CTCTGTGTAT GATCATCTGA
951 TGAGGTCCTG GGAATTACAG CACATGGATT CCTTTAAAC ACATCTCAAC
1001 CATACTCTT GGTAAATAAA AACATCTCTA ATTTGCTGTA ATTCACTATA
1051 ATGATATAAC AGCTATCCTG GAGTATTCCT GTGTCTAATT TCATGCTGGT
1101 AAAGCTCTGG TTATGGTACA ACAAAGATGA GGTAATTATT ACAACATCCT
1151 GCACATACTG GGGTATCTGT GGCATCCTTG GTACATCAGT CCTGAAACGA
1201 AGCCAATATC TACAGTAGCT TTGAGATGCG TAGGCGAGGG TAATTCTTTT
1251 ATGCTACTGA GGTGGTACTG TGTGGTCATT CTTTGTGATC TCCTGATGTT
1301 GCGATGCACA CCCACAAACA CACATTTGTA CACATATATT AATCATCAGG

1351 GCCATTATTA GCTCACAACA TTATCCTATC CTTCTTTTCT TCAATAACCT
1401 CTCCGAGTTT GAAGAGTCCA TGGCGATGAT TTGCGGGGTT TATACCTGTG
1451 ATTAAAGCGC ACACAAAAAA TGATATTGTG GAAAATAACA TGTCTTGTGA
1501 TCGAGCATGG CCAGCTGTAT AACTGTAAGA AGGATTAGAA CTGTGAATCA
1551 TCCTTAAGAA AAAAAAAAAA AAAAAAAAAAG CTAAATAAAT GCAATCTGCC
1601 CAAGAGGGAG GAAATGAATA CCTATAAACC ACAACTTCTA TCCAATCACA
1651 TACAGACAAA TCAGCCTTCA GACCAATCAA ACGTCTTCAT TTAAAGCTTA
1701 CCTGGACTTG GCATACTGCC CAGCTTTTCC AAAACTACTC ACAATAATAC
1751 CTTCAACAAC AGTTAAAAAA CGCTGGTACT CAAACAAAAT CAACAGCCTT
1801 TTCAACGACT GCTTTAAAAA AGACCAAACA AACAAACAAG GAACGTCTTA
1851 ACCCAGAGAA GACAATTGCT TGGGAGAGGA AAAGTTTGCT TCTGAGTTAG
1901 CAGCCTGTGG AAACAGGATT AGTGGGTGGG ATTGGGGTGT GCTCTGCCCA
1951 TAAATACAGG CTCAGCGCTG CGCTGGCACA CTGAGAAACT TGGACGGCAA
2001 CCCTTGCGGC TCACACAAAG CAGGAGGGTG GGAAGCCCAG GTAAGGCAAT

FIG. 8

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(57) Abstract: Methods of screening for agents for treating asthma are provided. The methods involve screening for agents that decrease the production or activity of a Gob-4 protein that has been discovered herein to play a role in producing the symptoms and pathological complications involved in asthma. Methods of treating asthma, as well as screening for and treating with inhibitors of a Gob-4 protein are also provided.



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INTERNATIONAL SEARCH REPORT

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B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/4 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US2003/0236220 A1 (ZHOU et al) 25 December 2003 (25.12.2005), see entire document.	1
A	KONDO et al. Interleukin-13 Induces Goblet Cell Differentiation in Primary Cell Culture from Guinea Pig Tacheal Epithelium. American Journal of Respiratory Cell and Molecular Biology. November 2002, Vol. 27, No. 5, pages 536-541, see entire article.	1
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Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230		Authorized officer Marianne DiBrino, Ph.D. <i>Marianne DiBrino</i> Telephone No. 571-272-1600

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Continuation of B. FIELDS SEARCHED Item 3:

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